BONE MARROW MORPHOLOGY IN MULTIPLE MYELOMA

DISSERTATION

SUBMITTED FOR

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THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

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Certificate

CERTIFICATE

This is to certify that the dissertation work entitled " **BONE MARROW MORPHOLOGY IN MULTIPLE MYELOMA**" submitted by Dr. Yegumuthu. K is the work done by her during the period of study in the department of Pathology, PSGIMS & R from June 2009 to April 2012. This work was done under the guidance of **Dr. Alamelu Jayaraman**, Professor and Head, Department of Pathology, PSGIMS & R.

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Introduction

INTRODUCTION

Multiple myeloma is a clonal plasma cell disorder that varies widely in its clinical course ranging from relatively indolent forms to frankly aggressive neoplasia [1]. The clinical manifestations of this disorder result from the proliferation and accumulation of these neoplastic cells and, the effect of marrow replacement by them. The pathologic manifestations are due to over production of certain proteins and constituent polypeptide chains. Bone marrow examination continues to be the cornerstone for establishing a diagnosis in association with other clinical and laboratory parameters [2]. Several clinical, laboratory and morphological variables help us in determining the prognosis of the disease. The first histological classification and staging of myeloma was put forth by Bartl et al in 1987[2]. Multiple myeloma has been classified into 6 histological types:

- Marschalko type
- Small cell type
- Cleaved type
- Polymorphous type
- Asynchronous type

- Blastic type

These six types were subsequently combined into 3 prognostic grades

- Low grade
- Intermediate grade
- High grade

Histological parameters that have a definitive prognostic significance in multiple myeloma are the percentage of myeloma cells in the marrow, pattern of infiltration, plasma cell atypia, marrow fibrosis & mitotic index[3].

The quantity of plasma cell burden in the biopsy has proved to be a very useful criterion for histological staging of multiple myeloma, supplementing any clinical staging system. Both these parameters, grade and stage provide information required for decisions on treatment modalities, while the effects of therapy can be monitored by sequential biopsies.

Classical cases of myeloma are composed of easily recognizable plasma cells and can be diagnosed without any difficulty.[4] Some cases may pose a considerable diagnostic problem as they exhibit unusual cytological features. This cytological aberration if unrecognized leads to an erroneous

diagnosis.[5] This study aims at analyzing the morphological variants of plasma cells in multiple myeloma.

Aims and objectives

AIMS AND OBJECTIVES

1. To analyze the occurrence of multiple myeloma in the bone marrow aspirate smears received between January 2005 to October 2011
2. To examine the bone marrow aspirate smears and perform a morphological grading of plasma cells
3. To analyze the percentage of myeloma cells in the marrow on aspirate smears
4. To review the bone marrow trephine biopsies using Bartl's histological grading system
5. To study the growth pattern and tumour cell burden in bone marrow biopsies

Materials and methods

MATERIALS AND METHODS

Patients who were clinically diagnosed as multiple myeloma in PSG OPD for whom bone marrow (BM) aspirate and trephine biopsy were done between January 2005 and October 2011, were included in the study.

SAMPLE COLLECTION:

After clinical evaluation, bone marrow aspirate and trephine biopsy were obtained for all patients. The trephine biopsy specimen was fixed in B5 solution for routine histopathological examination and the aspirate was smeared without any delay as the bone marrow clots faster than the peripheral blood.

MATERIALS REQUIRED FOR COLLECTION OF SPECIMEN:

- 70% alcohol or spirit swab for cleaning the skin
- Local anaesthetic agent – 2% xylocaine
- BM aspirate needle for obtaining BM sample
- Jamshidi needle for obtaining BM trephine biopsy
- Clean glass slides
- Methanol – fixative agent

- B5 Solution – fixative agent

BONE MARROW ASPIRATION PROCEDURE:

Satisfactory BM sample was generally aspirated from the sternum, iliac crest, anterior or posterior iliac spines. Both BM aspirate and trephine biopsy were obtained at a single sitting under local anaesthesia.

To perform marrow aspiration, the skin was cleaned with 70% alcohol and the skin, subcutis and overlying periosteum were infiltrated with local anaesthetic (2 – 5ml of 2% xylocaine). With a boring movement, the needle was introduced perpendicularly into the the ileum at the posterior superior iliac spine. After penetrating the bone, the stillete was removed and a 10 ml syringe was fixed to the needle to aspirate the marrow contents for making films.

The films were made without any delay. The films were made for a length of 3 – 5 cm from the aspirated marrow contents using a smooth edged glass spreader.

GIEMSA STAINING:

MATERIALS REQUIRED:

- Absolute methanol

- Giemsa stain [1 volume of stain with two volumes of distilled water]
- Mounting medium

STAINING PROCEDURE:

1. The slides were dried thoroughly. Fixation is for 20 minutes with absolute methanol
2. The slides were next exposed to Giemsa stain solution for 30 minutes
3. The stain was flushed with water
4. The back of the slides were cleaned with gauze and were allowed to air dry in a tilted position
5. The slides were mounted and labeled appropriately

EVALUATION OF BONE MARROW ASPIRATE SMEARS:

The smears were examined under scanner, low power, high power and oil immersion objectives. The preparation was considered satisfactory only when marrow particles and free marrow cells were observed in the stained films. A 500 cell differential count was performed in the cellular trails. In addition to the assessment of cellularity, evaluation of nature of erythropoiesis, assessment of myeloid series, the M/ E ratio, an estimate of

the number of megakaryocytes, any cytological or maturational abnormalities , the percentage of plasma cells in the aspirate was also estimated. All counted plasma cells were also subjected to morphological analysis.

BM TREPHINE BIOPSY PROCEDURE:

The trephine specimen was obtained from bone by using the Jamshidi needle. The needle was rotated to and fro to obtain a core of tissue. The specimen in B5 solution was transferred into 10% formalin after 2 – 4 hrs of B5 fixation. It was then decalcified in acid decalcifying agent (nitric acid 10 ml +distilled water 100 ml) and processed in an automated tissue processing unit before being embedded in paraffin. The sections were stained using routine hematoxylin and eosin, mounted and labeled.

HEMATOXYLIN AND EOSIN STAINING:

MATERIALS REQUIRED:

- Harris hematoxylin
- 1% eosin Y
- Xylene
- 1% acid alcohol

- Graded alcohol

STAINING PROCEDURE:

1. The sections were deparaffinized and hydrated through graded alcohols to water
2. Stained in Harris hematoxylin for 5 minutes
3. Washed well in running tap water until sections were blue for 5 minutes
4. Differentiated in 1% acid alcohol (1% hydrochloric acid in 70% alcohol) for 5 – 10 seconds
5. Washed well in tap water until sections were again blue (10 – 15 min)
6. Stained in 1% eosin Y for 2 minutes
7. Washed in running tap water for 1- 5 minutes
8. Dehydrated through alcohols, cleared in Xylene and mounted.

EVALUATION OF BONE MARROW BIOPSY SECTIONS:

Trephine biopsy was referred to as adequate if the trephine had at least 3 marrow spaces below the sub-cortical space. The biopsies were evaluated for cellularity, hematopoietic elements, volume and pattern of plasma cell

infiltration and degree of fibrosis. A morphological grading was done based on the criteria proposed by Bartl et al. In those cases where fibrosis was suspected a reticulin staining was performed.

GOMORI'S METHOD FOR RETICULIN STAINING:

MATERIALS REQUIRED:

- 0.5 % Potassium permanganate

  Potassium permanganate – 0.5 gm

  Distilled water – 100 ml

- 2% oxalic acid

  Oxalic acid - 2.0 gm

  Distilled water - 100ml

- 2% ferric ammonium sulphate

  Ferric ammonium sulphate 2.0 gm

  Distilled water – 100 ml

- 10% Silver nitrate solution

  2.5 ml of 10^5 aqueous solution of KOH was added to 10 ml of 10% silver nitrate solution and mixed well. 28% ammonia was added drop by drop until the precipitate was completely dissolved. 4 drops of silver nitrate was added again. The solution was made twice its volume by adding more distilled water.

- 20% formalin

  40% formalin - 20 ml

  Distilled water 100ml

PROCEDURE:

1. The sections were deparaffinized and brought to water
2. Treated with potassium permanganate solution for 1 minute
3. Washed with distilled water
4. Bleached in 2% oxalic acid solution for 1 minute
5. Washed in distilled water
6. Treated with 2% ferric ammonium sulphate for 1 minute
7. Washed in distilled water

8. Placed in a coplin jar of silver solution for 1 minute

9. Washed in distilled water

10. Reduced in 20% formalin for 1 minute

11. Rinsed in tap water

12. Dehydrated through graded alcohol, cleared in Xylene and mounted

GRADING OF FIBROSIS IS DONE AS FOLLOWS [6]

GRADING	DESCRIPTION
0	No reticulin fibres seen
1	Few scattered fine reticulin fibres
2	Network of fine reticulin fibres in most of the sections.Coarse reticulin fibres are absent
3	As in [2],with coarse reticulin fibres. Collagen fibres absent
4	Diffuse network of mainly coarse reticulin fibres.Collagen fibres present in some areas.

Review of literature

REVIEW OF LITERATURE

Multiple myeloma [plasma cell myeloma] is a differentiated B cell neoplasm characterized by skeletal dissemination of malignant plasma cells that produce monoclonal immunoglobulin [Ig] [7],[8]. Plasma cells are a crucial part of the immune system responsible for the production of antibodies in human beings. They are produced in the bone marrow and transported through the lymphatic system. Due to the fundamental nature of the system affected, multiple myeloma presents with heterogenous clinical features that makes it difficult to diagnose.

Multiple myeloma (MM) is a debilitating disease which has been present for decades and has gained the interests of physicians and scientists. Cases of possible multiple myeloma have been reported in American Indian skeletons from 200 AD. In 1845, Dr. William Macintyre described the features of myeloma in a patient named, Mc Bean. Macintyre and Bence Jones examined and described the properties of urine in that patient. Bence Jones estimated that 67gm of proteins / day was excreted by the patient and he concluded that the protein was 'hydrated deuteroxide of albumin'. Henceforth, the name Bence Jones protein came into existence.

In 1873, a Russian doctor, Dr.Von Rustizky performed an autopsy and found 8 separate tumors of the bone marrow which he designated as multiple myeloma [9],[10]. Hence, the term 'Rustizky's disease' is often used for MM in Russia. However, he did not mention about albuminuria.

Dr.Otto Kahler in 1885 noted one of the most striking cases of MM. The patient died two years later and the autopsy disclosed large round cells in the masses noted in the ribs and thoracic vertebra. Kahler recognized that the urinary protein had similar physical and chemical properties as described by Bence Jones. Dr.Otto Kahler reported this case in 1889 following which much interest was generated in this disease. Hence MM is also known as Kahler's disease named after him.

In 1956, Korngold and Lipari demonstrated a relationship between Bence Jones protein and serum proteins of MM. The designation of two major classes of Bence Jones proteins as Kappa and Lambda is a tribute to Korngold and Lipari [9],[10].

EPIDEMIOLOGY

Plasma cell myeloma comprises about 1% of all malignant tumours [11],[12] and accounts for 10 % of hematopoietic neoplasms [9],[13],[14]. MM is more common in men than in women. The incidence of myeloma

progressively increases with age. The median age at diagnosis is 70 years [15],[16]. Occurrence of myeloma in patients less than 40 years is rare. MM occurs more frequently in Afro – Carribean ethnic groups compared with Caucasians [17].

ETIOLOGY OF MUTIPLE MYELOMA

- Atomic bomb exposure

  Reports of increased incidence of myeloma among Japanese atomic bomb survivors have suggested an association between ionizing radiation and multiple myeloma.

- Occupational exposure

  The risk of myeloma is considered to be two folds higher in radiologists than in physicians who are not exposed to radiation. Agricultural workers, workers in metal occupation and industries, exposure to benzene have been reported to have an increased myeloma risk

- Lifestyle factors

  Dietary links: Higher risk of myeloma was found among people consuming large quantities of liver and butter. Decreased risks were

associated with the intake of cruciferous vegetables, fish and vitamin C supplements

Socio economic status: There exists an inverse relationship between the risk of multiple myeloma and socio economic status.

Hair dyes: Women with prolonged usage of hair dyes are prone for developing non Hodgkin's lymphoma and myeloma.

- Associated medical conditions: MGUS is a potential precursor for myeloma. The risk of progression is 1% per year[18],[19].

- Chronic antigenic stimulation: Myeloma is associated with chronic infections, inflammatory conditions, connective tissue disorders, auto immune illnesses, allergy related disorders and HIV.

PATHOGENESIS

Prolonged antigenic stimulations have been postulated in the pathogenesis of MM. A malignant stem cell in MM has not been identified, though phenotypic analysis has demonstrated involvement of early B lymphocytes circulating in the blood. These originate in the bone marrow, and / or in the lymphoid organs, circulate and seed in skeletal or extra skeletal sites. They use adhesion molecules to bind to the stromal elements

that provide the soil for their homing and maturation to mature plasma cells, which secrete a range of cytokines. Subsequent development and dissemination of MM is controlled by BM microenvironment which in turn is influenced by a battery of cytokines of which IL6 is the major myeloma factor. IL 6 appears to support the survival and expansion of myeloma cells by stimulating cell division and preventing programmed cell death.The net result of these cellular interactions determines growth and manifestations of MM. IL 6 along with IL 1 b and TNF alpha and other cytokines have osteoclastic activating properties and are responsible for lytic lesions involving the RANKL pathway [20],[21].

BONE MARROW MICROENVIRONMENT

MM is primarily a bone marrow disorder in which malignant monoclonal B cells differentiate into plasma cells. The circulating malignant B cells require an optimal environment for survival and differentiation. The malignant monoclonal B cells differentiate into plasma cells by interaction with stromal cells, endothelial cells, extracellular matrix and other malignant plasma cells. Adhesion molecules play a role in the connection and interaction of these cells and components. Myeloma cells express specific receptors for IL 6. IL 6 is produced by the cells in the BM microenvironment. Other cytokines like VEGF, IGF 1, SDF 1 and TNF

alpha are also produced. These cytokines determine the growth differentiation and adhesion of the tumour cells. Syndecan 1 is another important adhesion molecule in the myeloma cell [22]. A high level of syndecan 1 in the serum is an indicator of poor prognosis [23].

CLINICAL MANIFESTATIONS

MM is a well established clinical and immunological entity with considerable variability in biological behaviour and survival [2]. Majority of the myeloma patients are symptomatic at initial diagnosis presenting with bone pain [3], infections and anaemia [24] [25].

Bone pain usually involves the spine and ribs and worsens with activity. Persistent localized pain indicates a pathological bone fracture. Involvement of the vertebrae may lead to spinal cord compression. Bone lesions are lytic in nature and are best seen in plain radiographs, which shows punched out resorptive lesions (pepper pot appearance of the skull). Breakdown of bone can also lead to hypercalcemia.

The most common infections are pneumonias and pyelonephritis. Common pneumonia pathogens include S.Pneumonia, S.Aureus and K.Pneumonia. Common pathogens causing pyelonephritis include E.Coli

and other gram negative organisms. Increased risk of infection is due to immune deficiency resulting from diffuse hypogamma globulinaemia.

Renal failure may develop both acutely and chronically. It is commonly due to hypercalcemia but may also be due to Bence Jones protein excretion induced tubular damage. Other causes include glomerular deposition of amyloid, hyperuricaemia, recurrent infections and local infiltration of tumour cells.

Anaemia in myeloma is directly related to the percentage of plasma cell infiltrate in the marrow. In fact, absence of anaemia is considered to be a favourable prognostic factor [2]. Anaemia is usually normocytic and normochromic [9].

Common neurological manifestations due to hypercalcemia include weakness, confusion and fatigue. Other manifestations include radiculopathy, loss of bowel or bladder control (due to involvement of the spinal cord) and carpal tunnel syndrome (due to infiltration of peripheral nerves by Amyloid). It may give rise to paraplegia in late stages.

Patients with MM rarely live longer than 10 years [26]. A constellation of radiological, clinical, laboratory and pathological findings are combined to provide diagnostic criteria for plasma cell myeloma.

DIAGNOSTIC CRITERIA FOR MYELOMA [WHO 2001][27]

A. The diagnosis of myeloma requires a minimum of one major and one minor criteria or three minor criteria which must include 1 & 2.These criteria must manifest in a symptomatic patient with progressive disease.

B. MAJOR CRITERIA:

Marrow plasmacytosis [> 30 %]

Plasmacytoma on biopsy

M component [serum IgG >3.5gm / dl,IgA >3gm / dl, urine BJ protein > 1 gm / 24 hr]

C. MINOR CRITERIA:

Marrow plasmacytosis [10 – 30 %]

M component present but less than above

Lytic bone lesions

Reduced nomal immunoglobulins - < 50 % normal. [IgG < 600mg / dl, IgA <100mg / dl, IgM < 50 mg / dl]

DIAGNOSTIC CRITERIA FOR MYELOMA [WHO 2004][28]

SYMPTOMATIC PLASMA CELL MYELOMA

- M protein in serum or urine
- Bone marrow clonal plasma cells or plasmacytoma
- Related organ or tissue impairment

ASYMPTOMATIC MYELOMA

- M protein in serum at myeloma levels [30gm / l]
- Clonal plasma cells in the bone marrow [10 % or more]
- No related organ or tissue impairment

NOTE

- No level of serum or urine M protein is included [M protein in most cases is IgG > 30 g/l, IgA > 25 g / l, urine light chain > 1 gm/ 24hr]. But some patients with symptomatic myeloma have levels lower than these.
- Monoclonal plasma cells usually exceed 10% of nucleated cells in the marrow but no minimal level is designated. 5 % of patients with symptomatic myeloma have < 10 % marrow plasma cells

- Manifestations of end organ damage include anaemia, hypercalcemia, lytic bone lesions, renal insufficiency, hyperviscosity, amyloidosis and recurrent infections.

MYELOMA STAGING SYSTEM: SALMON AND DURIE'S CRITERIA

STAGE I:

- Low M protein levels [IgG < 50 g / l, IgA < 30 g/ l, urine BJ < 4 gm / 24 hrs]
- Absent or solitary bone lesion
- Normal Hb, serum Ca, Ig levels [non M protein]

STAGE II:

- Overall values between I and III

STAGE III:

- High M protein levels [Ig G >70 g / l, IgA > 50 g / l, urine BJ > 12 g / 24 hrs]
- Advanced multiple lytic bone lesions
- Hb < 8.5g / dl, serum Ca > 12 mg /dl

SUBCLASSIFICATION:

- A – Serum creatinine <2 mg /dl
- B - Serum creatinine >2 mg /dl

INTERNATIONAL STAGING SYSTEM:

STAGE I: Serum beta 2 microglobulin < 3.5 mg / l

Serum albumin > 3.5 g / l

STAGE II: Not stage I or III

STAGE III: Serum beta 2 microglobulin > 5.5 mg / l

BIOCHEMICAL FINDINGS

The serum protein electrophoretic pattern shows a peak or localized band in 80% of patients. Bence Jones protein is found in the urine in 75 % of patients. Quantitative measurements of the paraprotein are necessary to establish a diagnosis and to monitor the disease. Additional findings include elevated serum calcium levels due to osteolysis and elevated serum creatinine levels due to reduced renal function.

RADIOGRAPHIC FINDINGS:

The classic radiographic appearance of MM is that of multiple, well circumscribed, lytic, punched out, round lesions within the skull, spine and pelvis. Some patients may have diffuse osteopenia on radiography. Fewer than 10% of cases present with a single myelomatous lesion [plasmacytoma] which appear as bubbly expansions of a single bone, often the ribs or posterior elements of the spine. Radiographs of treated myeloma lesions may show areas of abnormal bone architecture with sclerosis. Around 79% of patients with myeloma demonstrate skeletal involvement.

HEMATOLOGIC FINDINGS

PERIPHERAL BLOOD

Rouleaux formation is usually the most striking feature on peripheral blood smears. A leukoerythroblastic reaction is observed in some cases. Plasma cells are found on peripheral blood smears in approximately 15 % of cases, usually in small numbers. Marked plasmacytosis i.e. if the number of clonal plasma cells exceeds 2 x10(9) / l or 20% of the leukocyte differential count, accompanies plasma cell leukemia [28].

BONE MARROW ASPIRATE

Myeloma plasma cells vary from mature forms to immature, plasmablastic and pleomorphic forms. The mature forms are indistinguishable from normal / reactive plasma cells. They are usually oval with a round eccentric nucleus and spoke wheel or clock face chromatin without nucleoli. The plasmablastic cells contain a centrally placed large hyperchromatic or vesicular nuclei with one or more prominent nucleoli and scanty cytoplasm [29], [30]. Various unusual cytological appearances of plasma cells seen in BM aspirates include

- Flaming cells – deep magenta to pink staining in the peripheral part of the cytoplasm
- Mott, morula, grape cells – cells with multiple small globular cytoplasmic inclusions
- Thesaurocytes – plasma cells with abundant reticulated cytoplasm
- Russell bodies – large intracytoplasmic globules of proteins
- Dutcher bodies – pale Intranuclear inclusions

These changes are not specific for myeloma since they may be found in reactive plasma cells. Other less known variants include cleaved cells,

pleomorphic variant, signet ring cells, histiocytoid cells, clear cells, spindle cells and oncocytic cells

BONE MARROW TREPHINE BIOPSY:

MM is a neoplasm of terminally differentiated B cells but its histological picture demonstrates the whole spectrum of B cell differentiation from small lymphocytes to plasma cells. The quantity of plasma cell burden in the biopsy proved to be useful criterion for histologic staging of multiple myeloma supplementing any clinical staging system in use.

The plasma cells were dispersed in the marrow and were also seen in small clusters in the paratrabecular and perivascular regions. Usually the residual hematopoietic marrow was decreased with maturation inhibition of erythropoiesis and a corresponding increase in fat cells. Even in minimal infiltrations some nucleolated plasma cells were identified as a reliable diagnostic parameter in early cases of multiple myeloma. The percentage of plasma cells with prominent nucleoli correlated with the density and amount of plasma cell burden.

Bartl et al compared the cellular size, cytoplasmic structure and nuclear configuration and concluded that the spectrum of myeloma cells could be divided into six groups:[3],[31],[32]

- Marschalko
- Small cell
- Cleaved
- Polymorphous
- Asynchronous
- Blastic

MARSCHALKO TYPE:

These plasma cells are akin to normal mature plasma cells. They have an eccentric cart wheel nuclei, perinuclear hof and basophilic cytoplasm. Nucleoli were noted in some and mitotic figures were observed. Their main growth pattern was interstitial.

SMALL CELL TYPE:

These plasma cells are small and round with a mean size of 13 mic. The nucleus with its dense chromatin resembles that of a small lymphocyte

[lymphoplasmacytoid]. A narrow rim of basophilic cytoplasm having a perinuclear hoff is noted. Nucleoli and mitosis are rare. Interstitial growth pattern was the predominant growth pattern.

CLEAVED TYPE:

Most plasma cells have notched, cleaved or even convoluted nuclei of variable size with intranuclear inclusions. There is a low nucleocytoplasmic ratio with a small perinuclear hof. The marrow was frequently packed in this type together with a marked fibrosis.

POLYMORPHOUS TYPE:

The predominant features include marked cellular pleomorphism, multinuclearity and giant plasma cells. Many plasma cells have cytoplasmic inclusions. Nucleolus is mostly prominent and centrally located. No predominant proliferation pattern was noted.

ASYNCHRONOUS TYPE:

This type is characterized by a marked asynchronous maturation of nucleus and cytoplasm. These cells have a large eccentric nucleus with prominent nucleoli, abundant basophilic cytoplasm and pronounced

perinuclear hof. Marschalko cells, lymphocytes and immunoblasts are scattered among the asynchronous cells forming nodules.

BLASTIC TYPE:

The infiltration consists of plasmablasts with large nuclei, vey prominent centrally located nucleoli with a moderate rim of basophilic cytoplasm and a faint perinuclear hof. Complete replacement of the bone marrow is typical.

PROGNOSTIC GRADING:

When the clinical course and the survival of these six histologic subtypes were compared, three major prognostic groups emerged.

- MULTIPLE MYELOMA OF LOW GRADE MALIGNANCY

  Comprises the Marschalko and small cell types

- MULTIPLE MYELOMA OF INTERMEDIATE GRADE MALINANCY

  Consists of the cleaved, polymorphous and asynchronous cell types

- MULTIPLE MYELOMA OF HIGH GRADE MALIGNANCY

  It represents the plasmablastic type

HISTOLOGIC STAGING OF MULTIPLE MYELOMA:[33]

Based on the plasma cell burden in the biopsy three stages are defined:

STAGE I: < 20% plasma cell mass

80% hematopoietic and fat cells

STAGE II: 20 – 50 % plasma cell mass

Variable amount of hematopoiesis and fat cells

STAGE III: > 50% plasma cell mass

Residual marrow consisted mainly of fat cells

HISTOLOGIC FACTORS PREDICTING UNFAVOUARABLE PROGNOSIS IN MYELOMA

- Nucleolated plasma cells
- Cleaved nuclei of plasma cells
- Large nuclei of plasma cells
- Mitotic figures of plasma cells
- Nodularity
- Packed marrow pattern

- High tumour cell burden
- Low ratio of hematopoiesis / fatty tissue
- Coarse fibrosis
- High osteoclastic index

IMMUNOPHENOTYPING

Immunohistochemistry is often fraught with difficulties as both non neoplastic and neoplastic plasma cells express various hematopoietic and non hematopoietic antigens.

Myeloma plasma cells usually express CD79a, CD 38, CD 138 and VS 38 C. In contrast to normal plasma cells, they are nearly always CD 19 negative. CD 56 is aberrantly expressed in many cases.[34],[35] Aberrant expression of CD 117, CD 20, CD 52 and CD 10 may be seen. Some cases are cyclin D1 positive.

Cytokeratin and epithelial membrane antigen positivity with leukocyte common antigen negativity could lead to an erroneous diagnosis of carcinoma.

DIAGNOSIS

Diagnosis is usually straight forward when the combination of diffuse osteoporotic or multiple osteolytic lesions, infiltration of the bone marrow with plasma cells and a serum or urine M protein is present [36].

- Reactive plasmacytosis of the bone marrow can usually be distinguished from myeloma by the polyclonal nature of the serum Ig and absence of monoclonal Bence Jones protein in the urine
- Osteolytic lesions can also be caused by metastatic cancer. Presence of osteosclerosis tends to differentiate such lesions from myeloma
- Benign MGUS is very difficult to be differentiated from myeloma because the difference is essentially a quantitative rather than qualitative nature. Distinction can be made by less level of paraprotein, less degree of Ig suppression, lack of progression, lack of injurious clinical manifestations.

TREATMENT

Although multiple myeloma is a well established clinical and immunological entity, progress in its treatment has been relatively slow. Partly it is due to difficulties associated with its biological behaviour.

Therapeutic interventions are required in patients with symptomatic and / or progressive myeloma. In general there are two sorts of therapy [37].

- Systemic therapy to control the progression of myeloma
- Symptomatic supportive care to prevent serious morbidity

Once a diagnosis of multiple myeloma has been made the initial standard treatment depends on whether the patient is a candidate for high dose chemotherapy with autologous stem cell transplant.

Following drugs are to be used in a transplant candidate

- High dose pulsed glucocorticoids
- VAD chemotherapy [vincristine 0.4 mg / d, doxorubicin 9mg/ m2/d, dexamethasone 40mg / day for 4 d / week]
- Thalidomide [200 mg] + dexamethasone 40 mg for 4 days every 2 weeks.

In patients who are not transplant candidates, the therapy consisted of

- Intermittent pulses of an alkylating agent, L-phenyl alanine mustard [LPAM melphalan] and prednisolone

Dose may need adjustments due to unpredictable absorption and differences in marrow tolerance.

Relapsed myeloma can be treated with novel agents including lenolidomide and / or bortezomib. These agents target the tumour cells, tumour cell – bone marrow interaction and the bone marrow milieu.

PROGNOSIS

The outcome in an individual patient with myeloma is profoundly influenced by a number of factors.

- Patients presenting with stage I disease have a longer median survival than stage III disease
- Significant irreversible renal failure is associated with poor prognosis
- Patients with beneficial response to therapy have a better prognosis than those who are refractory
- The extent of tumour burden bears an inverse relationship to survival
- Progressive disease may be obvious from worsening clinical manifestations

Currently available chemotherapeutic agents tend to reduce the bulk of myeloma tissue and prolong the survival but virtually will never eliminate the disorder.

The median overall survival of patients with myeloma is 7 – 8 years, with subsets of patients surviving over 10 years [38].

The major causes of death are progressive myeloma, renal failure, sepsis, therapy related acute leukemia or myelodysplasia. Nearly quarter of patients die of myocardial infarction, chronic lung disease, diabetes, stroke, all intercurrent illnesses related more to the age of the patient group than to the tumour.

Results

RESULTS AND ANALYSIS

The total number of biopsies received during the study period was 25,084

Total number of biopsies from the bone marrow: 976

Total number of bone marrow aspirate samples: 1663

Total number of cases diagnosed as multiple myeloma: 54

Of these 54 cases, only 34 patients underwent both bone marrow aspiration and biopsy procedure and they were included in the study. The remaining 20 cases for whom either aspirate sample or trephine biopsy were not done were excluded.

AGE DISTRIBUTION:

The mean age of the patients was 60 .6years (Range: 34 - 80 years) while all of them were more than 30 years Thirteen patients (38.2%) were between the age of 61 and 70 years and 21 patients were between 51 and 70 years. (Table 1) Majority of the patients were males (22/34 cases) with the male to female ratio of 1.8:1.

TABLE 1: AGE AND SEX DISTRIBUTION OF PATIENTS WITH MULTIPLE MYELOMA

AGE (YEARS)	NO. OF MALES (%)	NO. OF FEMALES (%)
31 - 40	2 (5.8)	0(0)
41 - 50	2 (5.8)	2 (5.8)
51 - 60	2 (5.8)	6 (17.6)
61 - 70	11 (32.3)	2 (5.8)
71 - 80	7 (20.5)	2 (5.8)
TOTAL	22	12

PERIPHERAL SMEAR FINDINGS IN PATIENTS WITH MULTIPLE MYELOMA:

TABLE 2: MORPHOLOGICAL PICTURE IN THE PERIPHERAL SMEAR

BLOOD PICTURE	NO. OF PATIENTS
NN (normocytic normochromic)	29
MN (macrocytic normochromic)	5
Total	34

As observed from table 2, NN picture was the most commonly observed peripheral smear pattern. Twenty patients showed exaggerated rouleaux formation in the peripheral smear and a bluish hue of the background was observed in 9 patients.

HEMOGLOBIN LEVELS: Of the study group, hemoglobin levels were normal only in three patients while the remaining 31 cases were anemic [91.1 %]. The patients who had normal hemoglobin levels had a NN blood picture in the peripheral smear. Four patients had hemoglobin levels less than 5 gm/dl (Table 3).

TABLE 3: DISTRIBUTION OF CASES BASED ON HEMOGLOBIN LEVELS

HB LEVEL	NO. OF PATIENTS
< 5 gm/dl	4
5- 10 gm/dl	21
10 – 12gm/dl	6
>12 gm/dl	3
Total	34

Erythrocyte sedimentation rate (ESR) was elevated in all patients with 17 of them having a value of more than 100 mm/hr. No significant

changes were observed in the morphological profile of leukocytes and platelets. Eight patients had leucopenia while three of them had leukocytosis. Thrombocytopenia was observed in 17 patients (50 %).

Occasional plasma cells were observed in the peripheral smear of one patient. A plasma cell count of 57 % was observed in another patient for whom a diagnosis of plasma cell leukemia was made.

TABLE 4: DISTRIBUTION OF CASES BASED ON % OF PLASMA CELLS

Percentage of plasma cells in peripheral smear	No. of patients
0%	32
1%	1
57%	1

Pancytopenia was observed in 5 patients, of which 2 patients each had MN anaemia and NN anaemia.The patient with plasma cell leukemia also presented with pancytopenia.

PATHOLOGICAL FINDINGS IN BONE MARROW ASPIRATE:

Hypercellular bone marrow was observed in all patients. . The erythroid, myeloid series and megakaryocytes were relatively suppressed. The morphology and percentage of plasma cell infiltrate in the bone marrow are well established prognostic factors. Plasma cell count of more than 50% was observed in twenty patients (Table 4).

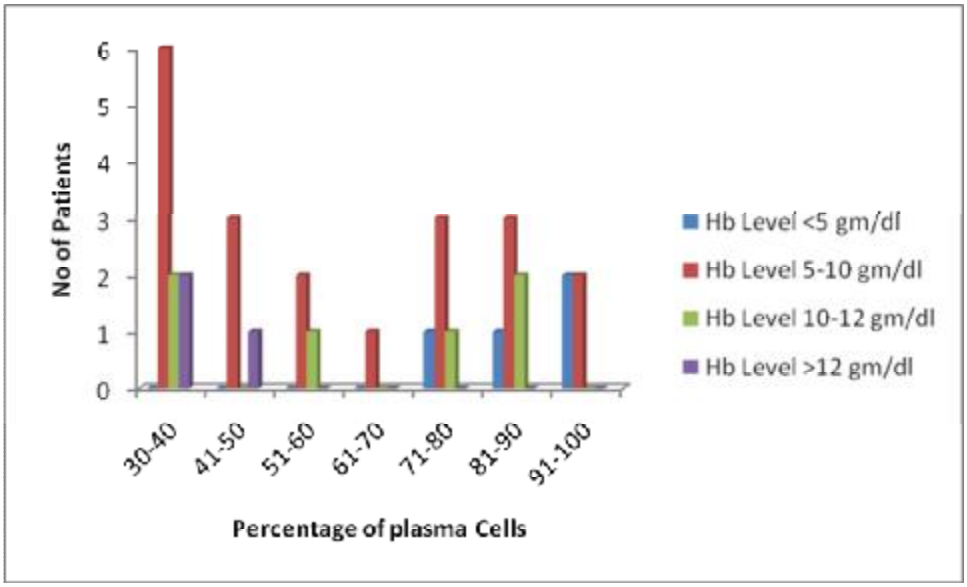
TABLE 5: PERCENTAGE OF PLASMA CELL INFILTRATE IN THE ASPIRATE SMEARS

% OF PLASMA CELLS	NO. OF PATIENTS	FREQUENCY (%)
30 - 40	10	29.4
41 - 50	4	11.7
51 - 60	4	11.7
61 - 70	1	2.9
71 - 80	5	14.7
81 - 90	6	17.6
91 - 100	4	11.7
TOTAL	34	

A mature plasma cell morphology was observed in 20 patients. These cells were spheroidal or ellipsoidal with eccentrically placed nucleus and abundant basophilic cytoplasm. The nuclear chromatin was dense with a perinuclear hof. Immature plasma cells with eccentrically placed relatively large nucleus, one or more nucleoli, diffuse chromatin pattern, a perinuclear clear zone and a variable amount of dense cytoplasm was observed in six patients. Plasma blasts with predominantly centrally placed large nucleus, round nucleoli and scant rim of cytoplasm were seen in eight cases.

Few plasma cells in the bone marrow aspirates had a characteristic appearance. Mott cells (morular or grape cells) were seen in one patient, flame cells in one and pale intranuclear inclusions called dutcher bodies in one patient. Binucleate and trinucleate plasma cells were observed in 26 cases (76.4%).

Fig 1 SUMMARIZES THE HEMOGLOBIN VALUES AND THE PLASMA CELL VOLUME IN THE BONE MARROW ASPIRATE



PATHOLOGICAL FINDINGS IN BONE MARROW TREPHINE BIOPSY:

Increased cellularity was observed in 32 patients while the remaining two had a normocellular marrow (Table 5).

TABLE 6: ASSESSMENT OF CELLULARITY

CELLULARITY	NO. OF PATIENTS
Hypercellular	32
Normocellular	2
Hypocellular	0
TOTAL	34

Complete marrow replacement was observed in 26 patients. The plasma cells were dispersed in the marrow in four patients and a nodular infiltration in individual marrow spaces was seen in four patients (Table 6). The residual hematopoietic marrow was generally decreased (Table 7).

TABLE 7: PATTERN OF INFILTRATION OF THE PLASMA CELLS

PATTERN	NO. OF PATIENTS
Nodular	4
Interstitial	4
Mixed	0
Diffuse	26
TOTAL	34

TABLE 8: IMPACT ON RESIDUAL HEMATOPOIESIS

HEMATOPOIESIS	NO. OF PATIENTS
Increased	0
Normal	2
Suppressed	32
TOTAL	34

Histologic staging of multiple myeloma was done based on the plasma cell burden in the biopsy as described by by Bartl et al (Table 8).

TABLE 9: HISTOLOGIC STAGING OF MULTIPLE MYELOMA

STAGE	PLASMA CELL BURDEN	NO. OF PATIENTS	FREQUENCY
I	< 20 %	0	0
II	20 – 50 %	8	23.5%
III	>50 %	26	76.4%

As observed from the table, majority of the patients were in the stage III (26/34) at the time of diagnosis. 76.4% of the patients had more than 50 % plasma cell infiltrate in the biopsy compared to only 58.6% in the aspirate indicating that the aspirate had underestimated the plasma cell burden. Fibrosis was not observed in any of the cases.

Histological grading of plasma cells was done based on Bartl's criteria (Table 9). Marschalko's types which are akin to normal plasma cells were the most commonly observed type. Cleaved cell, polymorphous and asynchronous morphology were not visualized in any patient.

TABLE 10: HISTOLOGICAL GRADING OF PLASMA CELLS

MORPHOLOGICAL SUBTYPE	NO.OF PATIENTS	FREQUENCY	HISTOLOGICAL GRADE
MARSCHALKO	22	64.7	low
SMALL TYPE	4	11.7	low
CLEAVED TYPE	0	0	Int
POLYMORPHOUS	0	0	Int
ASYNCHRONOUS	0	0	Int
BLASTIC	8	23.5	High

Analogous to the malignant lymphomas, Bartl et al has shown that multiple myeloma can also be divided into plasmacytic and plasmablastic morphology. For the purpose of analysis, well differentiated and intermediate type of myeloma cells were grouped together as plasmacytic type and poorly differentiated type of myeloma cells were taken as plasmablastic myeloma cells.

TABLE 11: COMPARISON OF TREPHINE BIOPSY FINDING WITH PLASMA CELL MORPHOLOGY

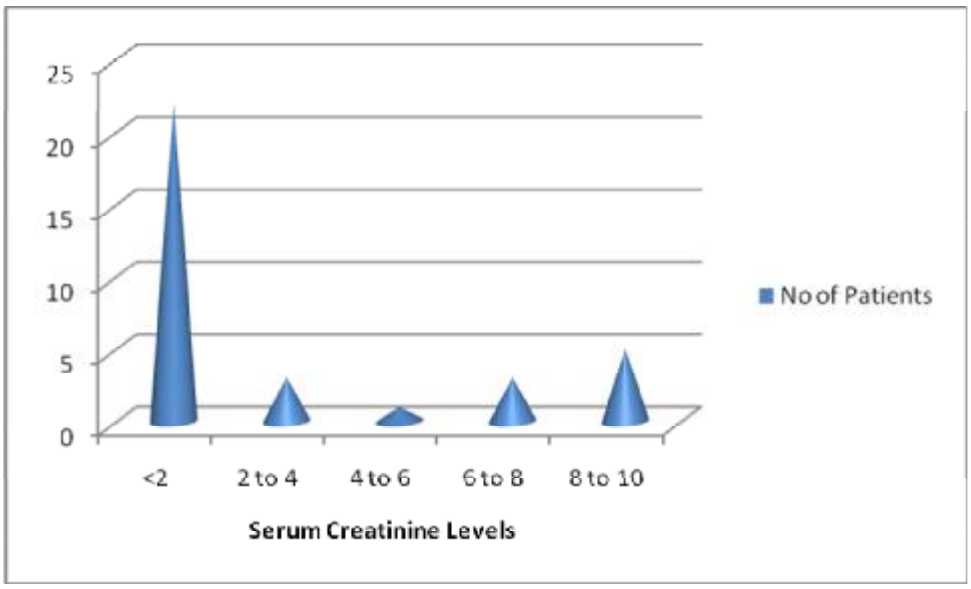
PARAMETERS		PLASMACYTIC	PLASMABLASTIC	TOTAL
VOLUME OF INFILTRATION	< 20%	0	0	34
	20 – 50 %	8	0	
	>50%	18	8	
PATTERN OF INFILTRATION	INTERSTITIAL	2	2	34
	NODULAR	4	0	
	DIFFUSE	20	6	
FIBROSIS	PRESENT	0	0	

Plasmacytic morphology was present in 26 cases and plasmablastic morphology was observed in eight patients. Plasma cell infiltration of more than 50% was observed in all cases with plasmablastic morphology. Histologically unfavourable features (plasmablastic morphology, > 50% infiltration and diffuse pattern of infiltration) were observed in six patients.

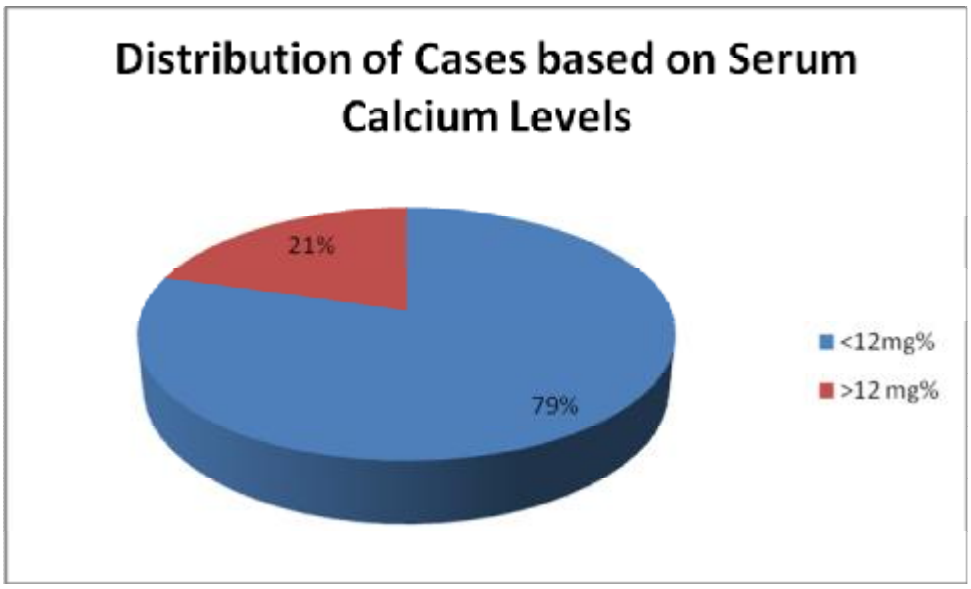
When a serum electrophoresis was performed, M band was demonstrated in 88.2% of cases.

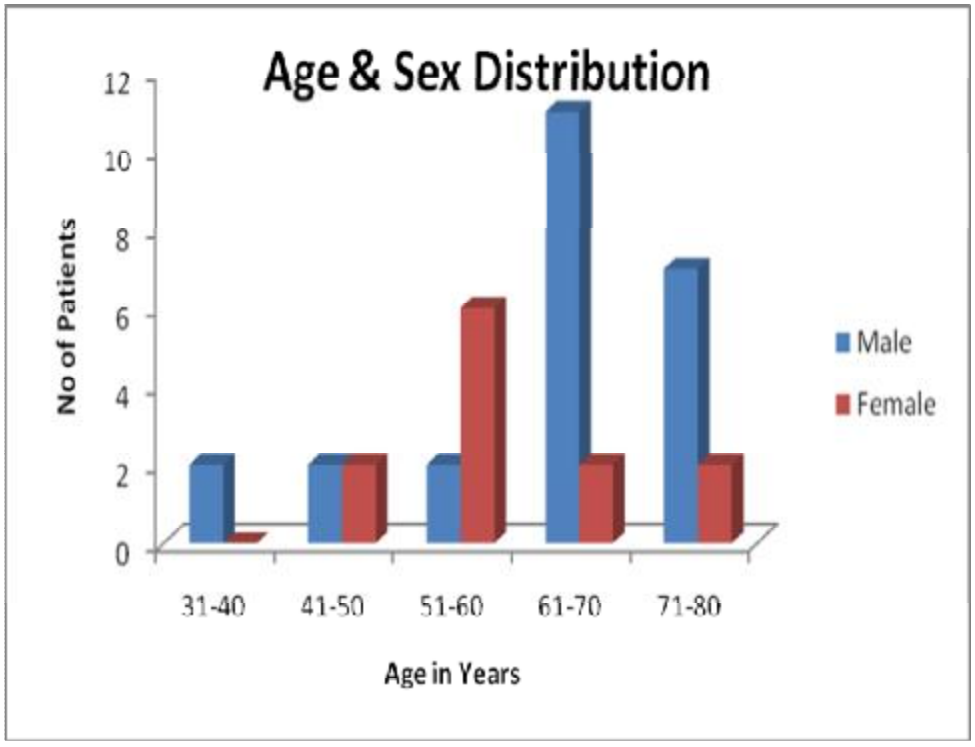
Elevated serum creatinine levels [2mg / dl] were noted in 12 / 34 cases

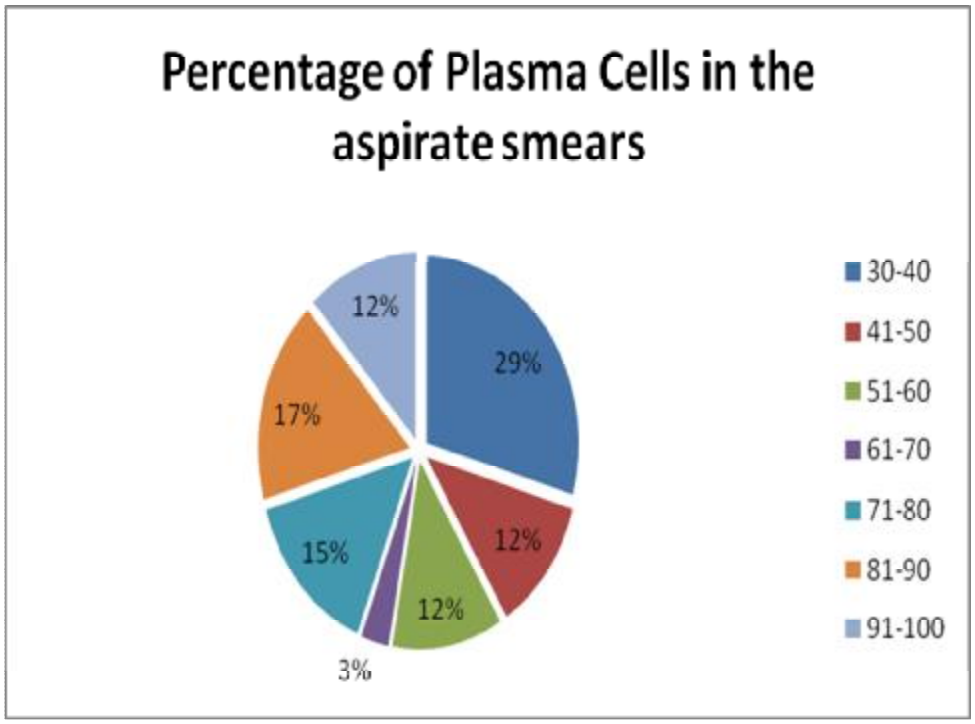
FIG 2: DISTRIBUTION OF CASES BASED ON SERUM CREATININE LEVELS



Larger percentage of cases [27 / 34 cases] had serum calcium levels less than 12 mg %




Age & Sex Distribution
No of Patients
12
10
8
6
4
2
0
31-40
41-50
51-60
61-70
71-80
Age in Years
Male
Female


Percentage of Plasma Cells in the aspirate smears
29%
12%
12%
3%
15%
17%
12%
30-40
41-50
51-60
61-70
71-80
81-90
91-100

Discussion

DISCUSSION

Multiple myeloma is a neoplastic disease of the bone marrow plasma cells causing lytic bone lesions, anaemia, renal insufficiency and hypercalcemia[39]. Despite the effectiveness of chemotherapy ,patients with multiple myeloma rarely live longer than 10 years. Kyle [1983] reported only 2 % of patients to survive for more than 10 years and Alexanian [1985] only 4% [40].

There have been very few studies from India [Carter et al, 1987, Terpstra et al 1992] which compared the value of bone marrow sections to smears. When a comparison was made between bone marrow aspirate and bone marrow biopsy procedures considerable discordance was observed in the volume of plasma cell infiltration in a high number of cases. The current study presents an approach to evaluate the bone marrow aspirate findings in multiple myeloma and to determine the value of bone marrow biopsy in establishing the diagnosis. All patients had undergone a bone marrow aspiration and biopsy at the time of diagnosis.

Our study showed a predominance of males with 22 /34 cases [64.7%] being men. Similar results were observed in previous studies [29][41].

The mean age of the patients was 60.6 years which is consistent with that of western literature and studies conducted in India [41], [42].

In our study there were 31 patients [91.1%] with anaemia. In a study conducted by Bartl et al, anaemia was found in 68% of cases where as in that which was conducted by Singhal et al, 30.6% of cases had anaemia.Peripheral smear examination showed that the anaemia was normocytic normochromic in nature in majority of the patients [29 cases]. In patients with multiple myeloma a dilutional effect of the expanded plasma volume with high concentrations of paraprotein contributes to lowered hemoglobin levels. Suppression of Erythropoiesis by bone marrow infiltration is an additional factor [43]. Other factors which contribute to anaemia are renal failure, chronic infection, bleeding and development of myelodysplastic or leukemic disorders.

20 cases showed exaggerated rouleaux formation in the peripheral smear. Increased rouleaux formation is usually seen at high concentrations of serum immunoglobulins [44]. Blood grouping and cross matching may be difficult because of red cell rouleaux formation.ESR was found to be elevated in all the patients.

One of the patients was diagnosed with plasma cell leukemia with a plasma cell count of 57% in the peripheral smear.

Leukocytopenia was observed in 8 patients and leucocytosis in 3 patients. There were 17 patients with thrombocytopenia. Pancytopenia was noted in a proportion of patients [5 / 34 cases]

It was observed in our study that those patients who had a hemoglobin level of < 5 gm % had greater than 80 % plasma cells on bone marrow aspirate smears. Studies by Subramanian et al have shown that hemoglobin levels correlate well with the percentage of plasma cells [2],[45]. In fact absence of anaemia was found to be a favourable prognostic factor.

In our study we found that, hypercellular marrow was observed in all 34 cases in the aspirate. 20 patients had a mature and 6 cases had a immature plasma cell morphology. 8 patients had a plasmablastic morphology. Studies by Philip kuriakose et al have shown a relationship between plasma cell morphology and survival [30], [46], [47], [48], [49]. Thus, they concluded that plasma cell morphology which is considered as a significant prognostic factor in multiple myeloma should be incorporated in the already existing clinical system.

Although bone marrow examination is a traditional and a rapid method for quantification of plasma cells, it is subjected to variability. This is due to technical problems like blood dilution or sample clotting. Variabilty in sampling is also due to focal disease distribution [50].Thus the exact extent of marrow involvement is better appreciated on a biopsy.

A histological classification and staging of myeloma based on bone marrow sections were proposed by Bartl and co workers. Important differences in survival were registered. But no comparison was made with the bone marrow smears. Very few studies compared the values of bone marrow sections to smears [51].

In the bone marrow trephine sections, the marrow was hypercellular in 32 patients. Usually the residual hematopoiesis was decreased. There were 32 patients with suppressed hematopoiesis. The normal hematopoietic reserve can be studied in bone marrow biopsy which is very essential for monitoring the therapy.

Subramanian et al had found that 71 % of patients had > 50 % plasma cells in the infiltrate in the biopsy compared to only 40 % in the aspirate. In the present study we found that 76.4 % of patients had more than 50 % infiltrate in the biopsy compared to 58.6 % in the aspirate.ie the aspirate had

underestimated the plasma cell burden. Similar observations were made by Terpstra et al as well [51], [52].

The tumour cell load is frequently underestimated when only a bone marrow aspirate has been performed. This is probably due to a mesh of reticulin fibres around the plasma cells in larger groups, which makes plasma cells resistant to aspiration. Thus a biopsy seems to be a more sensitive parameter for assessing the plasma cell burden.

Most cases of myelomas showed a diffuse pattern of infiltration in our study. [26/34 cases].In 4 patients each there was a nodular and interstitial pattern of infiltration. Interstitial pattern was the predominant pattern of infiltration in other studies [sailer et al, pich et al and bartl et al].

According to Stifter S et al, the type of infiltration pattern was in proportion with the stage of the disease. Interstitial and nodular patterns were observed when the hematopoiesis was still preserved. In contrast diffuse pattern of infiltration results in suppression of hematopoiesis [53].

According to Sailer et al, of all the histologic parameters the degree ofplasma cell differentiation is the most important one [54]. There were 26 cases with plasmacytic morphology and 6 cases with plasmablastic morphology. Studies by bartl et al and Subramanian et al have shown that

the plasma cell morphology has a good correlation with volume of infiltration, pattern of infiltration and degree of fibrosis. But in our study although 6/8 cases [75%] showed a diffuse pattern of infiltration, similar frequency [76%] was also observed in patients with plasmablastic morphology [20 / 26 cases].

The presence of fibrosis has been associated with a poor survival [54]. and it was not found in any of our cases. Fibrosis cannot be evaluated on aspirate smears. This again underlines the need to perform bone marrow biopsy in patients with multiple myeloma.

In our series we had [22 / 34] 64.7 % of patients with Marschalko, [4/ 34]11.75% of patients with small cell type and[8 / 34] 23.5 % of cases with blastic morphology. Recognition of morphological variants of plasma cells is necessary to avoid erroneous diagnosis. Presence of atypical forms should be reported in the pathological diagnosis because they are indicators of poor prognosis.

Different series have quoted that all the above mentioned histological parameters provide valuable prognostic information wherever other modalities like beta 2 microglobulin and IL6 are not available [33],[54].

According to G. merlini et al and Bartl et al the four clinical factors of prognostic significance are Hb level, M component in serum, serum creatinine levels and serum calcium levels [55],[56].

In our study we noted that 91.1% of cases had anaemia, 88.2% of cases had M component in serum, 38.8 %of cases had elevated serum creatinine levels, and 26.4% had Hypercalcemia. James H Jandl has hypothesized that, when activated by myeloma cytokines, osteoclasts proliferate, cling to the surfaces of bone and cause osteolysis with release of calcium. The major causes of renal failure are myeloma kidney [precipitation of monoclonal light chains in distal and collecting tubules] and hypercalcemia. Other causes include dehydration, Hyperuricemia and amyloidosis [9].

Owing to the improvements in therapeutic procedures, the survival has significantly improved in the past 5 years for patients with myeloma [9]. These advances coupled with remarkable strides in the understanding of the biology of the disease provide considerable hope and optimism for both patients and myeloma researchers.

Summary and conclusion

SUMMARY & CONCLUSIONS

Of the 1663 bone marrow aspirates that we received during the study period, in our laboratory there were 54 cases of multiple myeloma. The median age for diagnosis was 60.6 years with a male predominance. The percentage of plasma cells in the BMA smears varied from 30-95%. 58.8% of cases had a mature plasma cell morphology. In the trephine biopsy, the predominate pattern of infiltration was the diffuse pattern [76.4%] and Marschalko's type of plasma cells was the most commonly observed histological type [64.7%]. 23.5% of cases had a high grade malignancy as per Bartl's criteria.

Bone marrow examination is an essential investigation in the diagnosis of multiple myeloma. The plasma cell morphology at the time of diagnosis promises to be an important predictor of survival in patients with multiple myeloma. Therefore recognition of morphological variants of plasma cells is necessary to avoid erroneous diagnosis. Presence of atypical forms should be reported in the pathological diagnosis since they are indicators of poor prognosis.

In many cases the diagnosis is obvious from the bone marrow aspirate alone. Bone marrow aspiration is a traditional and rapid method for

quantification and morphological assessment of plasma cells, but it is subjected to variability due to blood dilution, sample clotting and focal disease distribution.

The factors which could be better judged in a trephine biopsy are, volume and pattern of infiltration which forms the basis for staging. Classification into low, intermediate and high grades based on histology, grading of fibrosis and plasma cell quantification by IHC are made possible in a trephine biopsy specimen. So, we recommend a bone marrow trephine biopsy in every case of suspected myeloma in addition to a bone marrow aspirate, because bone marrow trephine biopsy provides histologic parameters of prognostic significance.

In conclusion, a combined evaluation of bone marrow aspirate and trephine biopsy is superior to achieve more accurate and informative data, because they are each independently diagnostic.

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Master chart

S. No	Name	Age/sex	IP/OP. NO	PERIPHERAL SMEAR				Hb	BONE MARROW ASPIRATE FEATURES			BONE MARROW TREPHINE BIOPSY FINDINGS			BIOCHEMICAL FINDINGS		
				Rouleaux	RBC	WBC	Platelet		Hyper cellularity	Plasma cell %	Plasma Cell Morphology	Hyper cellularity	Pattern of infiltrate	Histological grading	Serum Creatinine	Serum Calcium	M band
1.	Lakshmi	55/ F	I05002076	+	MN	7.7	1.5	6.5	+	32	M	+	Dif	Mars	9.39	7.3	+
2.	Kesavan	72/ M	I05014587	+	NN	7.2	2.3	8.3	+	86	PB	+	Int	Blastic	0.7	8.5	+
3.	Paramasivan	63/ M	I05016401	_	NN	11.5	3.4	12.5	+	32	I	N	Int	Mars	0.8	9.0	_
4.	Kamalaveni	58/ F	I05022763	_	NN	4.6	2.4	10.7	+	90	PB	+	Dif	Blastic	0.76	7.2	+
5.	Palanisamy	73/ M	I06008798	_	MN	1.3	0.90	10.0	+	30	M	+	Nod	Mars	6.2	15.6	+
6.	Thiruvengadam	66/ M	I06034802	_	MN	14.7	1.6	9.3	+	33	M	+	Dif	Small	1.3	9.1	+
7.	Abdullakutty	61/ M	I06019385	+	MN	9.5	0.70	7.4	+	49	I	+	Dif	Mars	0.9	7.3	+
8.	Vasudevan	71/ M	I06023782	_	NN	4.9	2.75	11.1	+	55	PB	+	Dif	Blastic	0.9	8.0	_
9.	Sivabagyam	67/ F	I06027130	+	NN	5.9	0.66	8.6	+	73	PB	+	Dif	Blastic	0.7	10.4	+
10.	Ramaswamy	69/ M	I07003404	_	NN	7.0	2.4	12.5	+	50	I	+	Dif	Mars	1.45	8.3	+
11.	Arthanari	72/ M	I07031341	+	NN	8.2	2.11	6.7	+	67	PB	+	Dif	Blastic	1.2	12.0	+
12.	Palanisamy	70/M	I07036414	_	NN	4.2	1.24	6.4	+	48	M	+	Nod	Mars	8.4	8.7	+

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13.	Bagavath singh	63/ M	I08006790	+	NN	2.5	7.1	7.6	+	38	M	+	Dif	Mars	0.8	8.0	+
14.	Seetha	50/ F	I08007146	+	MN	2.4	1.34	7.6	+	55	M	+	Dif	Mars	7.88	7.3	+
15.	Palanivel	70/ M	I08008077	+	NN	8.2	1.16	6.7	+	87	M	+	Dif	Small	4.79	14.4	+
16.	Nachammal	75 /F	I08020524	+	NN	3.7	2.09	9.1	+	60	I	+	Dif	Mars	0.89	10.4	+
17.	Vijayalakshmi	53/ F	I08029598	+	NN	2.7	1.81	11. 3	+	32	M	+	Dif	Small	0.98	8.36	+
18.	Balu	63/ M	I08032743	+	NN	3.6	4.4	7.1	+	78	M	+	Dif	Mars	1.3	9.2	+
19.	Indrani	46/ F	I08045107	+	NN	6.2	1.35	4.2	+	93	M	+	Dif	Small	7.47	9.55	+
20.	Renganathan	45/ M	I08045309	_	NN	5.2	7.6	4.7	+	92	M	+	Dif	Mars	8.46	12.8	+
21.	Jaishankar	33/ M	I8048207	_	NN	7.0	1.22	6.1	+	94	M	+	Dif	Mars	1.52	8.79	_
22.	Raju	60/ M	I09045617	+	NN	7.9	1.90	9.3	+	75	I	+	Dif	Mars	0.95	9.0	+
23.	Pushpa Rengaraju	51/ F	I09026686	+	NN	7.9	1.75	9.8	+	95	PB	+	Int	Blastic	0.99	8.5	+

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				Rouleaux	RBC	WBC	Platelet		Hyper cellularity	Plasma cell %	Plasma Cell Morphology	Hyper cellularity	Pattern of infiltrate	Histological grading	Serum Creatinine	Serum Calcium	M band
24.	Jai Shankar	34/ M	I09042191	+	NN	6.2	1.16	9.1	+	82	M	+	Dif	Mars	2.3	8.15	+
25.	Abdul azeez	65/ M	I09041721	+	NN	11.6	0.95	7.7	+	50	PB	+	Dif	Blast	3.5	9.21	+
26.	Palanisamy	65/ M	I09044453	+	NN	5.5	1.52	9.9	+	55	M	+	Dif	Mars	0.94	7.2	+
27.	Muthammal	52/ F	I10013224	+	NN	3.2	1.87	8.0	+	40	M	+	Dif	Mars	0.7	9.1	+
28.	Ganesamoorthy	65/ M	I10018691	_	NN	5.9	2.2	5.7	+	32	M	+	Nod	Mars	8.2	12.3	+
29.	Govindammal	75/ F	I10034240	_	NN	14.9	1.27	9.9	+	35	M	N	Int	Mars	1.2	7.2	+
30.	Balan	48/ M	I10037556	_	NN	7.1	1.60	13.9	+	40	M	+	Nod	Mars	1.29	8.36	+
31.	Ramasamy	57/ M	I11014588	_	NN	14.9	3.65	11.5	+	85	M	+	Dif	Mars	0.76	9.1	+
32.	Dhanalakshmi	58/ F	I11015386	+	NN	4.7	0.92	11.0	+	80	I	+	Dif	Mars	1.45	15.6	+
33.	Duraisamy	74/ M	I11018831	_	NN	2.2	1.18	4.2	+	80	M	+	Dif	Mars	9.39	12.0	_
34.	Deivanayaki	64/ F	I11029029	+	NN	9.0	0.36	4.8	+	85	PB	+	Dif	Blastic	2.74	7.5	+

KEY TO MASTER CHART

- NN - NORMOCYTIC NORMOCHROMIC
- MN - MACROCYTIC NORMOCHROMIC
- M - MATURE
- I - IMMATURE
- PB - PLASMABLASTIC
- Dif - DIFFUSE
- Nod - NODULAR
- Int - INTERSTITIAL
- Mars - MARSCHALKO